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Differential Scanning Calorimetry Study on Thermal Denaturation of Human Carbonic Anhydrase II

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ABSTRACT: The thermal unfolding of human carbonic anhydrase II (HCAII) has been studied by circular dichroism, UV−vis spectrophotometry, and differential scanning calorimetry (DSC). Coincidence of aggregation and tertiary structure disruption as well as fitting of DSC data showed that a two-state model can properly explain thermal unfolding of HCAII. According to this model, the average values of T^* (the temperature at which $k = 1/60\ s^{-1}$), ΔH (enthalpy), and ΔE_a (activation energy) are equal to 335.8 K, 698.6 kJ·mol^{-1}, and 529.0 kJ·mol^{-1}, respectively.

INTRODUCTION

Protein folding and unfolding are one of the research fields of interest of scientists working in different areas. An investigation on this topic will pave the fundamental basis for understanding the protein structure−stability relationship and the factors affecting them. In the field of biotechnology, this knowledge can be applied to increase stability of biocatalysts and carrying out enzymatic process at higher temperatures. In medicine there are several diseases caused by protein misfolding. For example, marble brain syndrome (MBS) disease, known also as carbonic anhydrase II deficiency syndrome (CADS), can manifest in carriers of point mutations, His107Tyr substitution, in the human carbonic anhydrase II (HCAII) gene. Almstedt et al. demonstrated that this mutation has a remarkably destabilizing effect.[1]

For the first time, carbonic anhydrase (CA; carbonate hydrolyase, EC 4.2.1.1) was isolated by Margaria from ox blood in 1932.[2] CA is a zinc metalloenzyme catalyzing both phases of the reversible reaction $H_2CO_3 \leftrightarrows CO_2 + H_2O$; thus, it is physiologically important in the formation of CO_2 from bicarbonate in the lung. The various types of CA have been isolated from animal and plant cells and have been also found in some strains of the bacterial genus *Neisseria*. It has been shown that there exist five evolutionarily unrelated CA families named as α- (found in animals), β- (mostly found in higher plants), γ-CA (produced by the methanogenic archaebacterium, *Methunosarcina thermoghila*, when grown on acetate), δ-CAs (present in some marine diatoms), and ζ-CA (found in marine diatoms which contain Cd instead of Zn in their active site).[3] There are no significant sequence homologies between different CA families. In higher vertebrates, including humans, 16 different CA isozymes or CA-related proteins (CARP) have been described.[3,4]

In human, seven α-CA isoenzymes have been identified differing in their location in tissues and cells, catalytic efficiency, inhibitor binding, and gene sequence. Four types of them are cytosolic; HCAI, II, III, and VII. The isoenzyme with highest turnover rate, HCAII, is traditionally purified from red blood cells. HCAII plays an important role in bone, kidney, and brain functions so the HCAII deficiency may cause syndromes like osteoporosis, renal tubular acidosis, and cerebral calcification.[5,6]

X-ray crystallography studies showed that the active site forms a cavity almost in the center of the molecule. A zinc ion is located near the bottom of the cavity. Zinc ligands to three nitrogen atoms from His-94, His-96, and His-119 are in a tetrahedral geometry, with an H_2O or OH^-.[1,7]

Lavecchia and Zugaro carried out an experimental study on the thermal behavior of erythrocyte CA to estimate the thermodynamic parameters of the enzyme. They also studied the effects of thermal denaturation on the catalytic properties of the enzyme. According to their results, below 333 K the enzyme is very stable, whereas between (333 and 338) K a drastic decrease in the biological activity is observed.[8] They reported that the loss of HCAII activity is an irreversible process, while HCAII showed a reversible thermal unfolding using UV−vis spectrophotometry. They reported T_m, van't Hoff enthalpy (ΔH_{vH}), and ΔG at 298 K equal to 335.6 K, 1075 kJ·mol^{-1}, and 83.7 kJ·mol^{-1}, respectively. They described discrepancy in the reversibility seen in structural changes and thermal inactivation as the former reflects major structural changes, whereas the latter is related to local modifications of the active site region which cannot be properly rearrange upon cooling. In the other report by Matulis et al., represented thermograms of bovine CAII unfoldings in several buffers of

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